

## REMARKS

Claims 1-6, 11 and 12 are pending. Claims 1-6 and 12 have been amended. Claim 11 has been canceled without prejudice to the subject matter thereof. New claims 13 and 14 have been added. Support for the claim amendments and new claims may be found in the specification at, for example, at [0011]; [0012]; [0016]; [0017]; [0020]-[0024] (referring to Application Publication No. 2006/0099694); and Tables 1 and 2. Applicants respectfully submit that no new matter has been introduced by the amendments and their entry is respectfully requested.

### *Title*

The Examiner objected to the title because it recited “seaweed.” Applicants respectfully submit that the amendment to the title has obviated the objection.

The Examiner also objected to the specification as containing typographical errors. Applicants appreciate the Examiners attention to detail and have amended the specification to correct the typographical error.

### *Rejections under 35 USC § 112*

The Examiner rejected claims 1-6 and 11-12 under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner alleged that the specification lacks basis or support for “specifically producing” or “producing specifically” polyunsaturated acids from all possible diatomaceous algae. Applicants traverse the rejection, but in an effort to expedite prosecution have amended the claims to recite two specific families of algae, *Chaetoceros* and *Skeletonema* that are particularly discussed in the specification. Moreover, the term “specifically” has been deleted from the claims. Accordingly, Applicants respectfully request that this 35 U.S.C. § 112 rejection be withdrawn.

The Examiner rejected claims claims 1-6 and 11-12 under 35 U.S.C. § 112, second paragraph, alleging that the meaning of “producing specifically” is unclear. Applicants traverse the rejections, but in an effort to expedite prosecution have amended the claims to delete the term “specifically.” Hence, Applicants respectfully request that this 35 U.S.C. § 112 rejection be withdrawn.

### ***Rejections under 35 USC § 103***

The Examiner rejected claims 1-6 and 11 under 35 U.S.C. § 103(a) “as being unpatentable over McGinnis *et al.* taken with Reitan *et al.* (Journal of Phycology, Volume 30, Issue 6, Pages 972-979, 1994), and Dempster and Taguchi *et al.*”

Applicants respectfully traverse the rejection.

The claims are directed to methods for producing polyunsaturated fatty acids from cultures of diatomaceous alga, either *Chaetocerotaceae* or *Skeletonemaceae*, wherein at least one growth-limiting factor is applied at the end of the exponential growth phase of the culture, causing growth arrest and increased production of polyunsaturated fatty acids in the alga.

McGinnis refers to fatty acid production in algae following nitrogen deprivation during the **stationary** phase of culture growth. McGinnis states, “On day four, the cultures were subjected to nitrogen deprivation.” (page 20, col. 2) Referring to the growth curve presented by McGinnis in Figure 3 (reproduced below), it is clear that the **algal culture was in stationary phase on day four:**

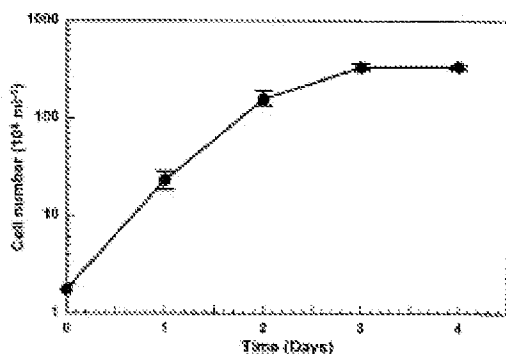


Figure 3. Typical growth curve for *C. muelleri* in Media Type II with a specific conductance of 25 mS cm<sup>-1</sup> and a temperature of 30 °C.

The bar graph cell growth presented in Figure 4 (reproduced below) also shows, even more specifically, that the algal cultures of McGinnis were well **passed the end of exponential growth phase when nitrogen deprivation was applied:**

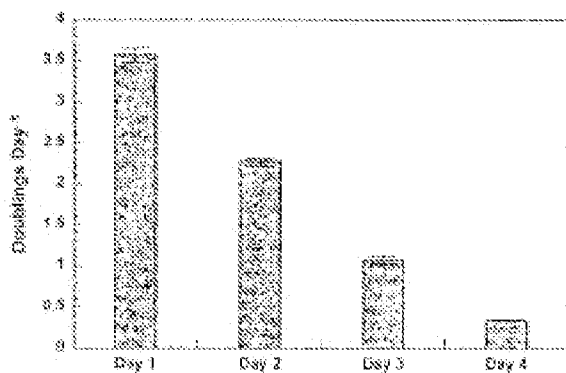


Figure 4. Growth of *C. muelleri* in doublings day<sup>-1</sup> in Media Type II with a specific conductance of 25 mS cm<sup>-1</sup> and a temperature of 30 °C.

The Examiner should note that the term “exponential growth phase” has a well-known meaning in the art as referring to the growth profile of a culture - not to the growth of any particular cell within that culture. Moreover, the claims specifically recite “culture” as opposed to “at least some diatoms were in exponential phase.” Thus, referring to McGinnis Figure 3, by day-two the culture had reached the peak of exponential growth, and by day-four (as indicated by the horizontal line evidencing that cell growth rate equaled cell death rate), the culture had passed into stationary phase. Indeed, McGinnis notes that “By Day 3, growth reached stationary phase.” (page 21, col. 1)

By contrast, as taught be the instant application and recited in the claims: “After 6-7 days ... the algae *cultures* were at the end of their exponential growth phase. Only at the end of the exponential growth phase were the algae stressed...” ([0016] emphasis added)

Finally, McGinnis teaches away from the claimed method. The claimed invention provides, advantageously, a source of Omega-3 polyunsaturated fatty acids. In contrast, McGinnis specifically refers to the increase in *triglycerides* in stressed *Chaetoceros* (page 23, col. 1), and concludes that it “a significant source of triglycerides.” (page 23, col. 2).

Reitan refers to culturing diatomaceous algae in various conditions for the analysis of polyunsaturated fatty acids. The Examiner asserts, referring to Table 2, that “the quantitative content of n-3 PUFAs increased with increasing growth limitation,” but this is not the conclusion of Reitan, which concludes that:

The percentage to total PUFAs *decreased* and the percentage of monounsaturated fatty acids increased *with the extent of nutrient limitation in all algal species*. ... *The*

***decreased relative content of PUFAs was mainly a result of a decrease in the sum of n-3 PUFAs.***

A closer examination of the individual PUFAs showed that ***the percentage of ... 22:6n-3 decreased*** in ... *Chaetoceros* ... with increasing extent of nutrient limitation... (page 974, col. 2 -page 975, col.1, emphasis added).

In contrast, the claimed method yields an ***increase*** in n-3 PUFAs. Moreover, although the effects of nutrient limitation were analyzed in Reiter, no particular limitations were induced at a particular point in culture growth phase. In sum, it is clear that Reiter has little bearing on the claimed invention.

Dempster refers to the diatom *Nitzschia communis*, and states that “growth rates were monitored during the first four days.” (page 10) Regarding nutrient deprivation, Dempster states:

The effects on lipid production from stress induced by the deprivation of the nitrogen source (urea) and stress caused by the natural depletion of the nitrogen source after the logarithmic growth phase (Day 4) of the experiment was determined ... The latter observations were used to determine the length of time necessary for Strain 3013 to naturally exhibit lipid yields comparable to that of the cultures subjected to artificial or premature nutrient depletion. (page 19)

The highest cell density achieved by Dempster was  $5 \times 10^5$  cells/mL (page 20). Regarding the lipid yield, Dempster reports, on page 33, that ***triglyceride*** (triolein equivalent) yield in media type Seri II was higher than that of in Seri I in both logarithmic phase or post-stress. Triglycerides are not omega-3 fatty acids. (see page 85) Further, Dempster reports that: “The ***highest*** average triolein equivalent lipid (NTE) yields were observed in the control ... and when the magnesium chloride concentration was ***increased***.” (page 68) Dempster also note, on page 83, that “Two green algae exhibited increased lipid content in response to nitrogen deficiency, whereas four blue-green algae did not ... whereas both increases and decrease were observed for diatoms after nitrogen deficiency ... lipid content fluctuated in *Ankistrodesmus*, *Chlorella* and *Isochrysis*.”

Regarding *Chaetoceros*, Dempster reports that ‘silicon deficiency’ resulted in an increase lipid synthesis rate of ‘1.7 times.’ (page 83-84) It is entirely unclear what lipid Dempster is referring to, but it should be noted that the following the claimed method Omega-3 synthesis increased almost 4 times after silicon deficiency in *Chaetoceros*. Finally, regarding *Nitzschia communis*, Dempster concluded that “Nitrogen deprivation stimulated a large increase in triglycerides, a small increase in sterols and fatty acids, and a decrease in cellular polar lipids.” (page 90)

As the Examiner acknowledged, the references differ from the claimed invention in that silicate deprivation is not disclosed and in that the growth arrest does not necessarily occur at about 6 or 7 days. This does not fully address the deficiencies of these references, however.

Taguchi refers to the natural shift in culture growth from exponential to stationary phase in a closed system (i.e., in which silicate was not replenished as media was spent) and the subsequent shift back to exponential phase when silicate was spiked into the media in cultures of *Chaetoceros gracilis*, *Hantzschia* and *Cyclotella*. This is not comparable to the claimed method in which silicate (or other nutrient) is specifically deprived at a particular, chosen point (the end of exponential growth). In other words, Taguchi studied growth boost, not growth arrest.

Additionally, Taguchi measured lipid content by relatively crude chloroform extraction (page 261), with no measurement of Omega-3 fatty acids. Accordingly it is no way certain that polyunsaturated fatty acids might be increased or decreased. Indeed, Taguchi states that “The general implication is that lipid synthesis can be triggered in diatoms by blocking cell division, and that the result in terms of lipid production is insensitive to the exact mechanism used to block division.” (page 265). The >100 page thesis by Dempster concludes otherwise.

Taguchi also teaches away from the claimed invention by reporting that “The increase in percent lipid carbon within one day of the onset of silicate limitation was the highest (3.6 times) for *Hantzschia* sp. and **lowest** (1.5 times) for *Chaetoceros*...” (page 265, emphasis added). In other words, why would one of ordinary skill in the art read Taguchi to suggest successful results with *Chaetoceros* instead of the much better *Hantzschia* when Taguchi teaches the opposite?

Further, that “the result in terms of lipid production is insensitive to the exact mechanism used to block division” teaches away from the claimed invention is borne out by the Applicants increased yield of Omega-3 fatty acids when the mechanism of nutrient deprivation is applied at the end of exponential phase.

On page 266, Taguchi lists approaches “necessary to harvest lipid-rich algae efficiently.” These approaches were compiled in **1987**, some **fifteen years** before the filing of the priority application. Far from rendering the claimed approach obvious, Taguchi evidences a long felt need to achieve what the present inventors have achieved.

The Federal Circuit has instructed that an obviousness is not supported by a combination of references that amount to hindsight or invitations to try:

In some cases, what would have been “obvious to try” would have been to *vary all parameters or to try each of numerous possible choices* until one possibly arrived at a successful result, *where the prior art gave either no indication of which parameters were critical or no direction as to which of many choices were likely to be successful*. ... In others what was “obvious to try” was to explore a new technology or *general approach* that seemed to be a promising field of experimentation, where the prior art gave *only general guidance* as to the *particular form* of the claimed invention or how to achieve it. *In re O’Farrell*, 7 USPQ2d 1673 (Fed. Cir. 1988) (emphasis added).

The cited references do not combine to provide for the claimed invention. For example, there is no indication that silicate deprivation initiated at the end of exponential growth phase is critical for the result of in an increase in Omega-3 fatty acid production in the claimed cultures of *Chaetocerotaceae* or *Skeletonemaceae* algae. The references are too vague, have too many variables, report conflicting results, and teach away from the claimed invention. Hence, Applicants respectfully request that this § 103 rejection be withdrawn.

### CONCLUSION

In view of the foregoing, Applicants respectfully submit that all the pending claims should be examined and are in condition for allowance. Early and favorable action is requested.

The Commissioner is hereby authorized to charge any fees, such as additional claims fees, and credit any overpayments that may be due in connection with this submission to Nixon Peabody LLP Deposit Account No. 50-0850.

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Respectfully submitted,

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